

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date
10 February 2005 (10.02.2005)

PCT

(10) International Publication Number
WO 2005/012495 A2

(51) International Patent Classification⁷:

C12N

(72) Inventors; and

(21) International Application Number:

PCT/US2004/024786

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(22) International Filing Date: 2 August 2004 (02.08.2004)

(25) Filing Language: English

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(26) Publication Language: English

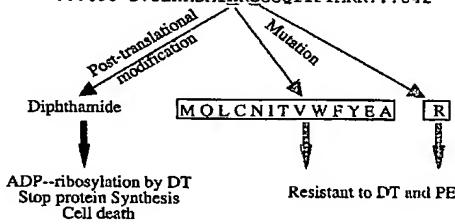
(30) Priority Data:
60/491,923 1 August 2003 (01.08.2003) US

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,

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(54) Title: METHODS FOR EXPRESSION AND PURIFICATION OF IMMUNOTOXINS

<i>H. sapiens</i> (66%)	...706	DVTLHADAIHRGGGQIIP	TARR...	858	SEQ ID NO:1
<i>M. musculus</i> (3'mRNA)	...	DVTLHADAIHRGGGQIIP	TARR...	...	SEQ ID NO:2
<i>R. norvegicus</i> (66%)	...706	DVTLHADAIHRGGGQIIP	TARR...	858	SEQ ID NO:3
<i>C. griseus</i> (CHO, 67%)	...706	DVTLHADAIHRGGGQIIP	TARR...	858	SEQ ID NO:4
<i>D. melanogaster</i> (67%)	...692	DVTLHADAIHRGGGQIIP	TARR...	848	SEQ ID NO:5
<i>C. elegans</i> (67%)	...700	DVTLHADAIHRGGGQIIP	TARR...	852	SEQ ID NO:6
<i>S. pombe</i> (79%)	...690	DVVLHADAIERGGGQIIP	TARR...	842	SEQ ID NO:7
<i>P. pastoris</i> (88%)	...690	DVTLHADAIHRGGQVIP	TMKRR...	842	SEQ ID NO:8
<i>S. cerevisiae</i>	...690	DVTLHADAIHRGGQIIP	TMRR...	842	SEQ ID NO:9



2070	2079	2088	2097	2106	2115	2124	2133
cGc cGc cGc							
GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA GGA CAA GTC ATT CCA ACC ATG AAG AGA							
D	V	T	L	H	A	D	A
690 700 710							
SEQ ID NO:10							

non-glycosylated immunotoxin containing eluant from the anion exchange column by eluting the non-glycosylated immunotoxin with a sodium borate solution; e) diluting the concentration of sodium borate in the second non-glycosylated immunotoxin containing eluant from step (d) to about 50 mM or less; f) concentrating the diluted non-glycosylated immunotoxin containing eluant from step (e) over an anion exchange column; and g) obtaining a purified non-glycosylated immunotoxin from the anion exchange column.

(57) Abstract: In one aspect the present invention relates to a method of expressing an immunotoxin in *Pichia pastoris* strain mutated to toxin resistance comprising a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract and maintaining a dissolved oxygen concentration at 40% and above; and b) performing methanol induction with a limited methanol feed of 0.5-0.75 ml/min/ 10L of initial volume during induction along with a continuous infusion of yeast extract at a temperature below 17.5 °C., antifoaming agent supplied up to 0.07%, agitation reduced to 400 RPM, and the induction phase extended out to 163 h. In another aspect, the present invention relates to a method of purifying a nonglycosylated immunotoxin comprising a) loading a solution containing the nonglycosylated immunotoxin onto a hydrophobic interaction column; b) obtaining a first non-glycosylated immunotoxin containing eluant from the hydrophobic interaction column; c) loading the non-glycosylated immunotoxin containing eluant from step (b) onto an anion exchange column; d) obtaining a second

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TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,

Published:

— *without international search report and to be republished upon receipt of that report*

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